Claims

1. A method for preparing a soluble protein comprising a modified form of urokinase-type plasminogen activator (uPA) or an active fragment thereof, or a variant of either of these which has uPA activity, which method comprises contacting said protein with a buffer at a pH of from 8.5-10.5, said buffer comprising a reducing agent and an oxidising agent which forms a redox pair, wherein the reducing agent is present in excess compared to the oxidising agent, and wherein the reducing agent is present in a concentration of at least 5 mM.

2. A method according to claim 1 wherein the protein is a non-native active fragment of urokinase-type plasminogen activator (uPA) or a variant thereof.

3. A method according to claim 1 or claim 2 wherein the protein is in uniformly stable isotope labelled form.

4. A method according to any one of the preceding claims wherein the buffer has a pH of from 9-10.

5. A method according to claim 4 wherein the buffer has a pH of 9.5.

6. A method according to any one of the preceding claims wherein the redox pair comprises reduced glutathione and oxidised glutathione.

7. A method according to any one of the preceding claims wherein the ratio of reducing agent: oxidising agent is at least 5:1.

8. A method according to claim 7 wherein the ratio of reducing agent: oxidising agent is in the range of from 5:1 to 15:1.

9. A method according to claim 8 wherein the ratio of reducing agent:oxidising agent is about 10:1.

10. A method according to any one of the preceding claims wherein the concentration of reducing agent is from 8mM-15mM.

11. A method according to claim 9 wherein the concentration of reducing agent is about 10mM.

12. A method according to any one of the preceding claims wherein the buffer comprises 50mM glycine, 10mM reduced glutathione (GSH), 1mM oxidised glutathione (GSSG).

13. A method according to any one of the preceding claims wherein the buffer further comprises one or more additives selected from non-detergent sulphobetaine (NDSB 201), arginine or salts thereof, L proline, 3-[{3-cholamidopropyl)dimethylammonio]1-propanesulfonate (Chaps) for example or lauryl maltoside.

14. A method according to claim 13 wherein the additive is non-detergent sulphobetaine (NDSB 201).

15. A method according to any one of the preceding claims wherein the urokinase-type plasminogen activator (uPA) is human uPA.

16. A method according to any one of the preceding claims wherein the protein is fused to an amino acid sequence which is useful in purification of the protein.

17. A method according to claim 16 wherein the protein of SEQ ID NO 2:

SEQ ID NO 2

1 hhhhhhrsaq sgqktlrprf kiiggeftti enqpwfaaiy rrhrggsvty

51 vcggslispc wvisathcfi dypkkedyiv ylgrsrlnsn tqgemkfeve

101 nlilhkdysa dtlahhndia llkirskegr caqpsrtiqt islpsmyndp

151 qfgtsceitg fgkenstdyl ypeqlkmtvv klishrecqq phyygsevtt

201 kmlcaadpqw ktdscqgdsg gplvcslqgr mtltgivswg rgcalkdkpg

251 vytrvshflp wirshtkeen glal

18. A method according to any one of the preceding claims wherein, in a preliminary step, the protein is denatured.

19. A method according to claim 18 wherein the denaturation is effected using 8N urea or 6M guanidine hydrochloride.

20. A method according to claim 16 or claim 17 wherein the protein product is subjected to a subsequent plasmin digestion step.

21. A method according to any one of the preceding claims wherein the protein is recombinant modified uPA or an active fragment thereof, or a variant of any of these, which has been expressed in a transformed host cell.

22. A method according to claim 21 wherein the host cell is a bacterial cell.

23. A method according to claim 22 wherein the protein is recovered from inclusion bodies in the host cell.

24. A method according to claim 22 or claim 23 wherein the host cell is transformed with a nucleic acid which encodes said protein, and wherein at least some of the codons present in the wild-type sequence of the nucleic acid are modified so that they are optimised for expression in a bacterial cell.

25. A method for preparing a soluble protein comprising uPA or an active fragment, or variant of any of these which has uPA activity, said method comprising transforming a bacterial host cell with a nucleic acid which encodes said protein, culturing transformed cells, isolating protein from inclusion bodies within the cells, denaturing the protein in solution in a buffer, and precipitating the protein from a buffer having a pH of from 8.5 to 9.5, said buffer comprising a reducing agent and an oxidising agent which forms a redox pair, wherein the reducing agent is present in excess compared to the oxidising agent, and wherein the reducing agent is present in a concentration of at least 5mM.

26. A method according to claim 25 wherein the product is subjected to a plasmin digestion to form an active fragment of uPA.

27. Soluble protein comprising modified uPA or an active fragment, or variant of any of these which has uPA activity, obtainable by a method according to any one of the preceding claims.

28. Protein according to claim 27 which has been uniformly (≥98%) isotopically labelled with ^{15}N and has a ^{15}N-^{1}H TROSY-HSQC NMR spectrum as shown in Figure 1B, when measured in a buffer of 50 mM HEPES, pH 7.3 at a temperature of 303 K.

29. Protein according to claim 28 wherein the isotopic labelling comprises ^{15}N or ^{13}C or any combination of these nuclei with ^{2}H.

30. A method for identifying ligands for uPA, said method comprising carrying out an analysis by NMR, isothermal titration calorimetry or differential scanning calorimetry on protein according to any one of claims 27 to 29, in the presence of test compounds, provided that in the case of NMR, the material is suitably labelled.

31. A method according to claim 30 for identifying ligands for uPA, said method comprising carrying out an analysis by NMR, wherein the protein is in uniformly stable isotope labelled form.

32. The use of protein according to any one of claims 27 to 29 for carrying out analysis by X ray crystallography.